Lassa Virus Infection of Rhesus Monkeys: Pathogenesis and Treatment with Ribavirin

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Rhesus monkeys were experimentally infected with Lassa virus to establish their suita-
bility as a nonhuman primate model for the human disease and to test the protective ef-
ficacy of ribavirin, an antiviral drug. Six of 10 untreated control monkeys died after
subcutaneous inoculation of $10^{4.1}$ plaque-forming units of Lassa virus (strain Josiah).
Infectivity titrations of tissue homogenates from the six dead monkeys indicated signifi-
cant replication in all tissues tested except the central nervous system. This distribution
of virus was confirmed by direct immunofluorescence examination of cryostat-sec-
tioned tissues. Ribavirin was beneficial in the treatment of two groups of infected
monkeys. Four monkeys first treated on the day of viral inoculation experienced only
mild clinical disease; four monkeys first treated five days later experienced a more
severe illness. None of the eight monkeys treated with ribavirin died. Viremia titers and
elevations of levels of serum transaminases in treated monkeys were significantly lower
than in controls. Ribavirin may be beneficial in the treatment of humans exposed to this
life-threatening virus.

Human infection with Lassa virus is a severe, gen-
eralized disease described as Lassa fever [1–3].
Since the initial observation in 1969 of the disease
in a missionary nurse in Nigeria, four outbreaks in
that country have been reported [3]. Subsequent-
ly, outbreaks or recognized instances of endemic
illness have been reported from Liberia and Sierra
Leone; serologic data also suggest the presence of
Lassa virus in Guinea and Senegal [2]. High fatali-
ty rates (20%–40%) have been reported for this
disease in hospitalized patients, although serologic
data have suggested that mild or subclinical infec-
tions may occur [2]. Treatment of infected patients
has been largely symptomatic and supportive; spe-
cific treatment has been attempted in a small
number of patients to whom Lassa-immune plas-
ma was administered, with equivocal success [4].
Management of Lassa fever would be facilitated if
an effective antiviral drug were available. More-
over, the number of patients might be substantially
reduced if a vaccine or antiviral drug were avail-
able to protect hospital and laboratory workers
who are at high risk of severe infection. However,
progress in all areas of research on Lassa virus has
been slow because this highly pathogenic virus
may be handled safely only within maximal con-
tainment facilities.

We report progress on development of a rhesus
monkey model for human Lassa fever. The results
are encouraging, suggesting that ribavirin, a drug
with broad antiviral activity against RNA and
DNA viruses [5, 6], is effective in treating severe
Lassa virus disease in rhesus monkeys. Ribavirin
reportedly has an acceptable margin of safety,
even in treated human patients with viral infec-
tions less severe than Lassa [7]. From the numer-
ous clinical trials reported [8–10], the principal
side effects appear to be reversible anemia and
transiently elevated bilirubin levels. The present
studies suggest that ribavirin may be of significant
value in treating persons soon after known ex-

posure to Lassa virus. The development of a rhesus monkey model for human Lassa disease will also facilitate the testing of other antiviral agents, experimental vaccines, and immuno-therapy procedures for efficacy in the management of this life-threatening disease.

Materials and Methods

Lassa fever virus, strain Josiah, was isolated in 1976 from the serum of a 40-year-old man in Sierra Leone [11]. This isolate was passaged four times in monolayer cultures of Vero cells (an African green monkey kidney cell line). This stock viral suspension contained 2.5×10^7 pfu/ml, and was stored at -70 C until it was diluted for use.

Infectious Lassa virus was assayed by counting pfu on Vero cells. Volumes (0.2 ml) of decimal dilutions of virus suspended in Eagle's minimal essential medium with Earle's salts and nonessential amino acids (EMEM) plus 2% fetal calf serum (FCS) were adsorbed to Vero cell monolayers grown in 10-cm² wells of plastic plates for 60 min at 37 C in 5% CO_2. After adsorption, the cells in each well were overlaid with 2 ml of medium containing Eagle's basal medium with Earle's salts, 5% FCS, HEPES buffer (25 mM), 50 µg of genta-micin/ml, and 1% agarose (Sea-Kem®; Marine Colloids, Rockland, Me.) and incubated for three days at 37 C in a humidified atmosphere containing 5% CO_2. After incubation, 2 ml of neutral red (Grand Island Biological Co., Grand Island, N.Y.), diluted in Puck's saline A to a final concentration of 1:6,000, was added. Plaques were counted after an additional incubation period of 18–24 hr.

Ribavirin. The antiviral drug ribavirin (Vira-zole®; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carbox-amide) was supplied by Mr. W. Cooley, ICN Pharmaceuticals, Covina, Calif. Stock solutions of ribavirin in water were prepared (100 mg/ml), and appropriate volumes were injected into monkeys (see below) or diluted in EMEM for in vitro experiments.

In vitro ribavirin sensitivity assays. Alveolar macrophages were recovered from rhesus monkeys by the method of Kastello et al. [12]. These cells were selected because they support replication of Lassa virus in the intact animal; also, they were obtainable as a relatively homogenous cell population that may be maintained in primary cell culture. Cells were suspended at a concentration

of 2×10^6/ml in EMEM with 10% FCS plus 50 µg of gentamicin/ml (growth medium) in 10-cm² wells of plastic plates and were allowed to attach for 2–3 hr at 37 C in 5% CO_2. Nonadherent cells were removed by aspiration.

Vero cell cultures were prepared in parallel. Cell monolayers were 50%–80% confluent when used, 4–5 hr after seeding. Lassa virus was diluted in EMEM with 10% FCS; 0.2-ml volumes were adsorbed to cells for 1 hr at 37 C in 5% CO_2. After adsorption, the residual inoculum was removed by aspiration, and the monolayers were washed once with 2 ml of growth medium. Finally, 2 ml of growth medium containing various concentrations of ribavirin (0, 1, 10, or 50 µg/ml) was added, and an 0.2-ml sample was immediately removed to determine residual inoculum concentrations at time 0. Each combination of virus and drug concentration was tested in triplicate. After incubation for four days, supernatant fluids were assayed for infectious virus, and cells were examined by a direct immunofluorescence test (see below) to detect Lassa viral antigens.

Inoculation and treatment of monkeys. Eighteen fully conditioned, young adult monkeys (*Macaca mulatta*), weighing 4–7 kg, were caged individually in a maximal containment laboratory. All monkeys were inoculated sc with 0.5 ml of stock Lassa virus diluted in EMEM to a concentration of 1.2×10^6 pfu/0.5 ml. Four monkeys were treated with ribavirin sc, beginning immediately after viral inoculation on day 0; four other monkeys received the initial ribavirin treatment five days after viral inoculation. All ribavirin-treated monkeys received a loading dose of 50 mg/kg, followed by injections of 10 mg/kg at 8-hr intervals until day 18. The dose regimen for ribavirin was selected on the basis of experience with efficacies of graded doses of the drug in the treatment of influenza in mice [13] and the extension of these studies to monkeys [14]. In clinical trials, the dosage employed has usually approximated 15 mg/kg per day. Unpublished data from this laboratory using [¹⁴C]ribavirin have suggested that the regimen we selected produces concentrations of drug between 1.2 and 4.5 µg/ml in plasma and between 30 and 50 µg/g of liver tissue (L. Brennecke, personal communication). Ten monkeys served as infected, untreated controls.

To obtain blood, monkeys were lightly anesthetized with ketamine and bled by femoral venipuncture; Vacutainer clot tubes (Becton-Dickinson,

Rutherford, N.J.) were used for virus and antibody studies, and heparinized tubes were used for hematologic studies. Monkeys were observed daily, but infections were permitted to follow their natural course without intervention except for injection of ribavirin. Complete necropsies werê performed within several hours of death.

Processing of tissues. For infectivity assays, portions of tissues were removed aseptically, weighed, and ground with mortar and pestle with Alundum® in 5 ml of EMEM and 10% FCS. It was not feasible to perfuse tissues from dead animals before this procedure. Tissue homogenates were centrifuged at 10,000 g for 15 min at 5 C. Supernatants were stored at −70 C until assayed for virus.

Tissues prepared for immunofluorescence were embedded in a polyethylene glycol compound (O.C.T.®; Ames Co., Elkhart, Ind.) and frozen at −70 C until cut in a cryostat. Tissues for standard histologic examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Total leukocyte concentrations in heparinized blood were determined with the aid of a Coulter counter (Coulter Electronics, Hialeah, Fla.). For platelet counts, blood was diluted with the Unopette® system (Becton-Dickinson) and counted in a hemacytometer. Transaminase levels were measured using Statzyme® kits for serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (Worthington Diagnostics, Freehold, N.J.). All studies with unfixed tissue or fluid were performed in maximal containment facilities.

Immunofluorescence. For detection of Lassa viral antigens in cells and tissue sections, a direct fluorescent antibody (DFA) technique was used. Hyperimmune antiserum to Lassa virus was prepared by sc inoculation of guinea pigs with 10^3 pfu of Lassa virus, followed by sc inoculation of 10^6 pfu 30 days later. Two weeks after the second inoculation, guinea pigs were bled for serum; the globulin fraction was precipitated with methanol and conjugated with fluorescein isothiocyanate (FITC) by standard procedures [15]. The conjugate was used at a dilution of 1:20 and had an FITC:protein ratio of 1.13:1.

Frozen sections, 6-μm thick, or cell suspensions evaporated to dryness on glass slides were fixed in acetone at room temperature (about 24 C) for 10 min, treated with conjugate for 30 min in a hu-

midified chamber at room temperature, washed in phosphate-buffered saline (PBS), pH 7.4, for 10 min, and mounted in phosphate-buffered glycerol, pH 7.4. Slides were examined with a Leitz microscope (Dialux®; Wetzler, West Germany) equipped with dry objectives and an incident mercury light source. Controls routinely included uninfected cells and tissues treated with FITC–guinea pig antibody to Lassa virus and infected tissues treated with FITC-conjugated antibodies prepared against serologically unrelated viruses. During standardization, inhibition or "blocking" controls were used. All controls were unremarkable.

For antibody assays an indirect immunofluorescence assay (IFA) was performed by the procedure of Peters et al. [16], with slight modifications. In brief, Lassa virus–infected or control Vero cell suspensions were dried onto circular areas of microscope slides coated with Teflon® (Cel-line Associates, Minotola, N.J.), fixed in acetone, and treated with test serum diluted in PBS. After incubation at room temperature for 30 min, slides were washed in PBS for 30 min, treated with FITC-conjugated goat antiserum raised to monkey γ-globulin (prepared as described above) for 30 min, washed, mounted, and examined. The end point was the highest dilution of serum producing definite, granular fluorescence confined to the cytoplasm of infected cells. End points were confirmed by independent examination of the slides by two investigators.

Results

Sensitivity of Lassa viral replication to ribavirin in vitro. Ribavirin inhibited the replication of Lassa virus in the two types of primate cells tested (table 1). The degree of inhibition was influenced by drug concentration, viral multiplicity of infection, and cell type. In Vero cells inoculated with 7.3 $\log_{10}$ pfu (corresponding to a $\log_{10}$ multiplicity of infection of 1.3), the highest drug concentration tested (50 μg/ml) had a negligible effect on viral replication. However, significant inhibition was observed at this drug concentration when lower input multiplicities were used. To demonstrate an inhibitory activity of ribavirin at lower concentrations in Vero cells, very low input multiplicities of Lassa virus were required.

In contrast, the antiviral activity of ribavirin was more pronounced when tested in rhesus alveolar macrophages. In these cells, ribavirin, 10

Table 1. Effect of ribavirin on replication of Lassa virus in cultured cells tested four days after inoculation.

Cells, $\log_{10}$ MOI*	Residual inoculum at time 0	Infectivity ($\log_{10}$ pfu/ml)[†] Ribavirin (μg/ml)				Percentage positive by direct fluorescent antibody assay after ribavirin (μg/ml)			
		0	1	10	50	0	1	10	50
Vero									
+1.3	2.3	5.3	5.2	5.4	4.8	100[‡]	100[‡]	100[‡]	100[‡]
−1.3	0.3	6.2	6.3	6.4	3.9	100[‡]	100[‡]	100[‡]	5
−3.3	<	7.0	6.8	5.8	<	100[‡]	100[‡]	100	0
−5.3	<	6.0	6.2	3.1	<	100	60	5	0
−6.3	<	5.6	<	<	<	75	0	0	0
Rhesus alveolar macrophages									
+1.3	2.6	5.2	5.2	1.7	1.5	100	80	0	0
−1.3	0.6	5.0	4.7	<	<	40	20	0	0
−3.3	<	3.6	2.8	<	<	1	0	0	0
−5.3	<	3.5	<	<	<	1	0	0	0

* MOI (multiplicity of infection) = $\log_{10}$ Vero pfu − ($\log_{10}$ cell number/well).

[†] Geometric mean titer for three determinations; < = <0.3 $\log_{10}$ pfu/ml.

[‡] Significant CPE, with most cells detached from monolayer.

μg/ml, completely inhibited viral replication, even at the highest multiplicity tested. (Virus recovered from the supernatant of cultures inoculated at high multiplicity probably represented residual inoculum.)

Confirmatory data were provided by examination of ribavirin-treated cells for viral antigens, with use of immunofluorescence. The proportions of cells containing viral antigens were dramatically reduced in the treated cultures with decreased yields of infectious virus. This reduction would suggest that ribavirin inhibited replication of Lassa virus at an early stage, before the accumulation of viral antigens within cells.

Ribavirin treatment of Lassa virus–infected rhesus monkeys. To establish base-line virologic and clinical data, 10 rhesus monkeys were inoculated sc with 6.1 $\log_{10}$ pfu of Lassa virus. Six of the 10 monkeys died, between 10 and 14 days after viral inoculation. All monkeys were viremic by day 5 (figure 1). The six control monkeys that eventually died developed significantly ($P < 0.001$) higher viremia titers than those that survived (figure 1). Viremia titers in all lethally infected monkeys exceeded 4 $\log_{10}$ pfu/ml, whereas none of the four surviving monkeys ever developed titers in excess of this apparently critical value.

The six lethally infected control monkeys were clinically ill by day 7. Characteristically, they remained huddled in the corners of their cages, exhibited a severe petechial rash most apparent on the face, were anorectic, and often developed hiccups when disturbed. Rectal temperatures remained normal or slightly elevated until several hours before death, when they fell precipitiously. The four surviving control monkeys also became clinically ill; one monkey experienced only a mild illness, but the remaining three were severely ill. These surviving monkeys developed anorexia, facial rashes, and bleeding from the gums and nares; one developed a cough. They recovered slowly over a period of three weeks and developed
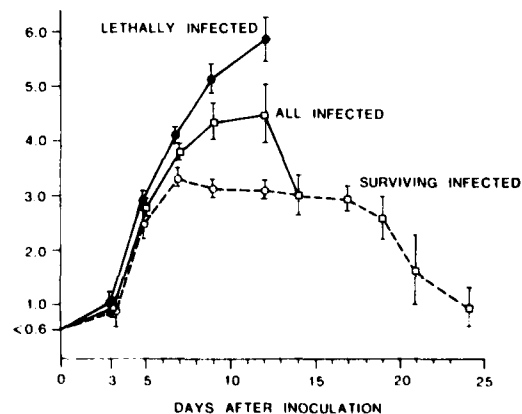

Figure 1. Lassa fever viremia, given in $\log_{10}$ pfu/ml of serum, in untreated rhesus monkeys. Points are geometric mean titers ± SE. Data for lethally infected (●) are based on six monkeys; for surviving infected (○), on four monkeys; and for all infected (□), on 10 monkeys.

no obvious sequelae in the ensuing seven-month observation period.

In contrast to untreated controls, none of eight monkeys treated with ribavirin died. Four monkeys were treated with ribavirin (50 mg/kg loading dose, followed by 10 mg/kg, three times a day), beginning immediately after viral inoculation (day 0) and continuing through day 18. In this group of monkeys, the onset of detectable viremia was delayed until day 7, and peak viremia titers were significantly lower than those of surviving control monkeys (figure 2). In a second group of treated monkeys, ribavirin therapy was delayed until day 5 (after the onset of viremia); all four of these monkeys survived. Viremia titers remained relatively low and never exceeded 4 $\log_{10}$ pfu/ml.

Clinical illness was mild and brief in the group that received ribavirin initially on day 0. Two of four monkeys exhibited no clinical signs at all; the
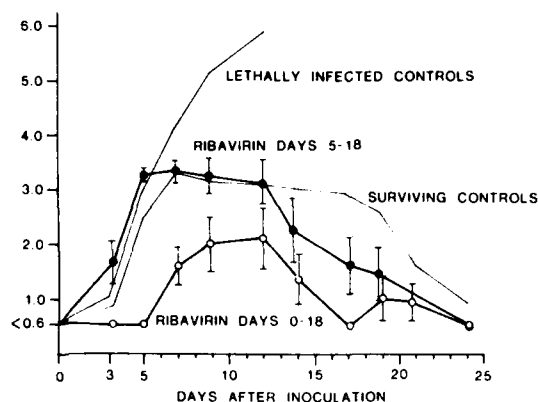

Figure 2. Effect of ribavirin on Lassa fever viremia, given in $\log_{10}$ pfu/ml of serum. Groups of four monkeys received ribavirin initially on day 0 (O) or day 5 (●) after inoculation with Lassa virus. Points are geometric mean titers ± SE. Curves for lethally infected and surviving controls are those from figure 1.
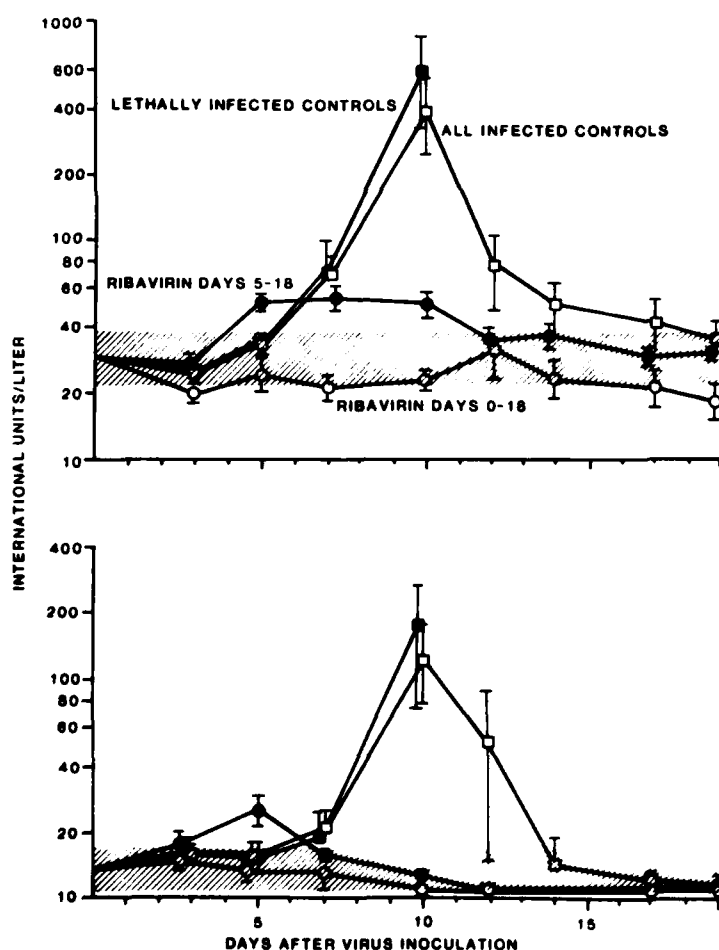

Figure 3. Serum glutamic oxalo-acetic transaminase (*top*) and serum glutamic pyruvic transaminase (*bottom*) activities in sera from control and ribavirin-treated monkeys infected with Lassa virus. Points are arithmetic means ± SE, based on the same monkeys whose viremia titers were presented in figures 1 and 2.

remaining two became only slightly depressed, and developed a minimal facial rash during the second week. One of these monkeys had hiccups from day 32 to day 34 and subsequently appeared to be blind on days 38–40. The four monkeys receiving ribavirin first on day 5 experienced a moderately severe disease course. All huddled and became anorectic, like untreated controls, between days 7 and 10. They became increasingly depressed after day 10 and developed moderately severe facial rashes. One developed a cough that persisted from day 14 until day 38. A second treated monkey appeared to be blind from day 32 through day 38. However, all four monkeys treated therapeutically with ribavirin eventually recovered, with no evident sequelae.

Hematologic and clinical laboratory studies. Serum transaminase activities were compared for infected control monkeys vs. ribavirin-treated monkeys (figure 3). Both SGOT and SGPT activities were markedly elevated in the infected controls between days 7 and 12, with peak values attained on day 10 (SGOT mean, 400 international units/liter; SGPT mean, 123 international units/ liter). In contrast, transaminase activities were only slightly elevated in the group treated with ribavirin, and fluctuations were insignificant in monkeys receiving ribavirin initially on day 0.

Analysis of transaminase data was of limited value in predicting the outcome of infection. Mean enzyme activities for lethally infected monkeys as a group were not significantly higher than mean values for all control monkeys. Two of the four surviving monkeys developed peak SGOT values of 400–500 international units/liter on day 10, although two lethally infected monkeys had peak SGOT values of only 245 and 139 international units/liter. Similarly, SGPT activities did not correlate closely with lethality in untreated, infected monkeys. However, since ribavirin-treated monkeys never developed marked elevations in transaminase activities, measurement of these enzymes might be useful in monitoring the efficacy of drug treatment.

White blood cells and platelets were also counted on sequentially obtained blood from four infected control and four treated monkeys. Transient and modest leukopenia was observed in both groups, with minimal mean counts of 3,100 white blood cells/mm^3 on day 7. Platelet counts did not fluctuate significantly. As with the transaminase data, there was no strict correlation between fluctuation

in the measured parameters and lethality. Similarly, serum concentrations of Na$^+$ and K$^+$ did not fluctuate significantly in these monkeys. Larger numbers of animals will be required before the prognostic value of such measurements can be assessed.

Antibody responses to Lassa infection in rhesus monkeys. The serologic responses, as measured by IFA, of ribavirin-treated and infected control monkeys were compared (figure 4) with use of a FITC-conjugated antiserum raised against monkey IgG, IgA, and IgM. Antibodies were first detectable on day 10 in the sera of all three groups of monkeys. In monkeys receiving ribavirin initially on day 0, the antibody responses were slightly suppressed but eventually reached titers similar to those of the other groups. There was no suggestion from these data that ribavirin exerted any immunosuppressive effect.

All control monkeys, including those that died, developed IFA titers by day 10. The geometric mean IFA titer for the six lethally infected monkeys on day 10 was 1:79 (range, 1:10–1:2,560), and for the four surviving controls, 1:266 (range, 1:160–1:640). The biologic importance, if any, of this statistically significant ($P < 0.05$) dif-
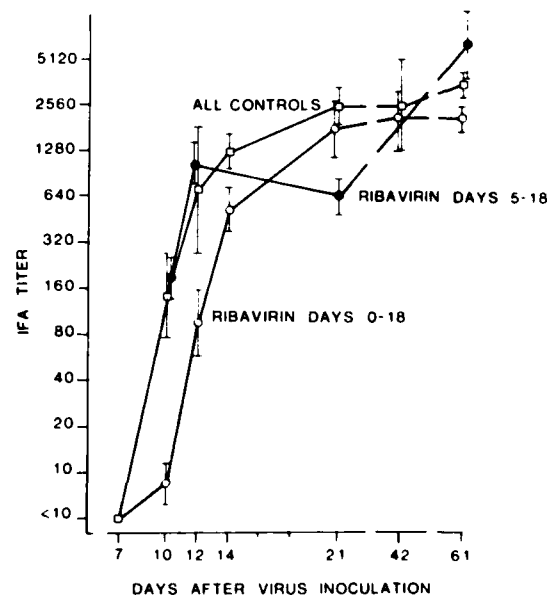

Figure 4. Titers of antibody to Lassa virus in sera from infected control and ribavirin-treated monkeys, assayed by the indirect fluorescent antibody (IFA) technique. Points are geometric mean titers ± SE, based on the same sera assessed in figures 1–3.
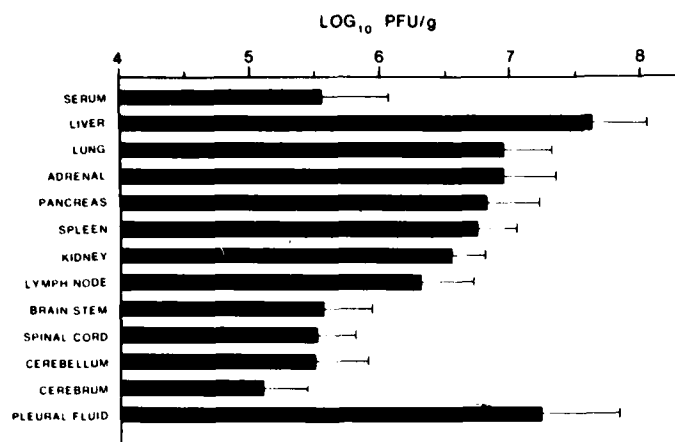
LOG$_{10}$ PFU/g

Figure 5. Concentrations of infectious Lassa virus recovered from tissues of lethally infected, control rhesus monkeys. Titers are geometric means ± SE, based on tissues from six monkeys.

ference in mean antibody titers remains to be determined. It is clear that in all groups, high titers of IFA antibody were detectable when serum concentrations of virus were close to their peak values, and that substantial viral concentrations were maintained in serum of surviving monkeys for one week or more after the appearance of IFA titers.

Distribution of Lassa virus in lethally infected rhesus monkeys. The concentrations of infectious virus recovered from tissues of the six lethally infected control monkeys were determined (figure 5). The livers contained the highest concentrations of virus (mean titer, 7.6 log$_{10}$ pfu/g); virus was also recovered, in titers significantly higher than those in blood, from all visceral tissues tested, including lung, adrenal glands, pancreas, spleen, kidney, and lymph nodes. In contrast to visceral tissues, central nervous system tissues contained lower viral concentrations than the blood; thus, brain stem, spinal cord, cerebellum, and cerebrum did not appear to be major sites of viral replication, although the possibility of minimal replication of virus in critical sites (such as vascular endothelium) could not be excluded.

Frozen sections of the tissues titrated in figure 5 were stained to detect Lassa viral antigens by the DFA technique. A detailed correlation of infectivity titers, distribution of viral antigens, and development of histologic lesions will be presented separately,[1] but typical distributions of fluorescence and the corresponding histologic lesions are

[1] R. T. Callis, A. De Paoli, and P. B. Jahrling, "Pathology of Lassa Virus in the Rhesus Monkey," manuscript in preparation.

shown in figure 6. In liver, various degrees of hepatocellular necrosis were seen (figure 6, upper left), usually accompanied by a slight infiltration of inflammatory cells. By the DFA technique, individual cells contained large quantities of Lassa viral antigens (figure 6, upper right). The majority of these cells were clearly hepatocytes (based on sizes and shapes), but the possibility of infection of Kupffer cells could not be excluded. Infected hepatocytes were randomly distributed throughout the parenchyma. In the adrenals, intense fluorescence was routinely observed in epithelial cells of the zona glomerulosa, extending into the epithelial cords of the zona fasciculata (figure 6, middle left). No corresponding lesions were detected in conventional histologic sections. Lung tissue typically contained patchy areas of interstitial pneumonia (figure 6, middle). By the DFA technique, cells in the thickened alveolar septae and free macrophages in the alveolar spaces appeared to be infected (figure 6, middle right).

Lymph nodes typically contained large infected cells, probably macrophages or reticuloendothelial cells, associated with the sinuses (figure 6, lower left). Similarly in the spleen, large infected cells were observed in the red pulp, but viral antigens were largely excluded from white pulp (figure 6, lower right). In tissues of the central nervous system, viral antigens were rarely detected except for occasional individual cells in the vicinity of small blood vessels. Thus these observations by the DFA technique confirmed the conclusion from infectivity titrations that Lassa virus replicated in most visceral tissues but was largely excluded from central nervous system tissues.
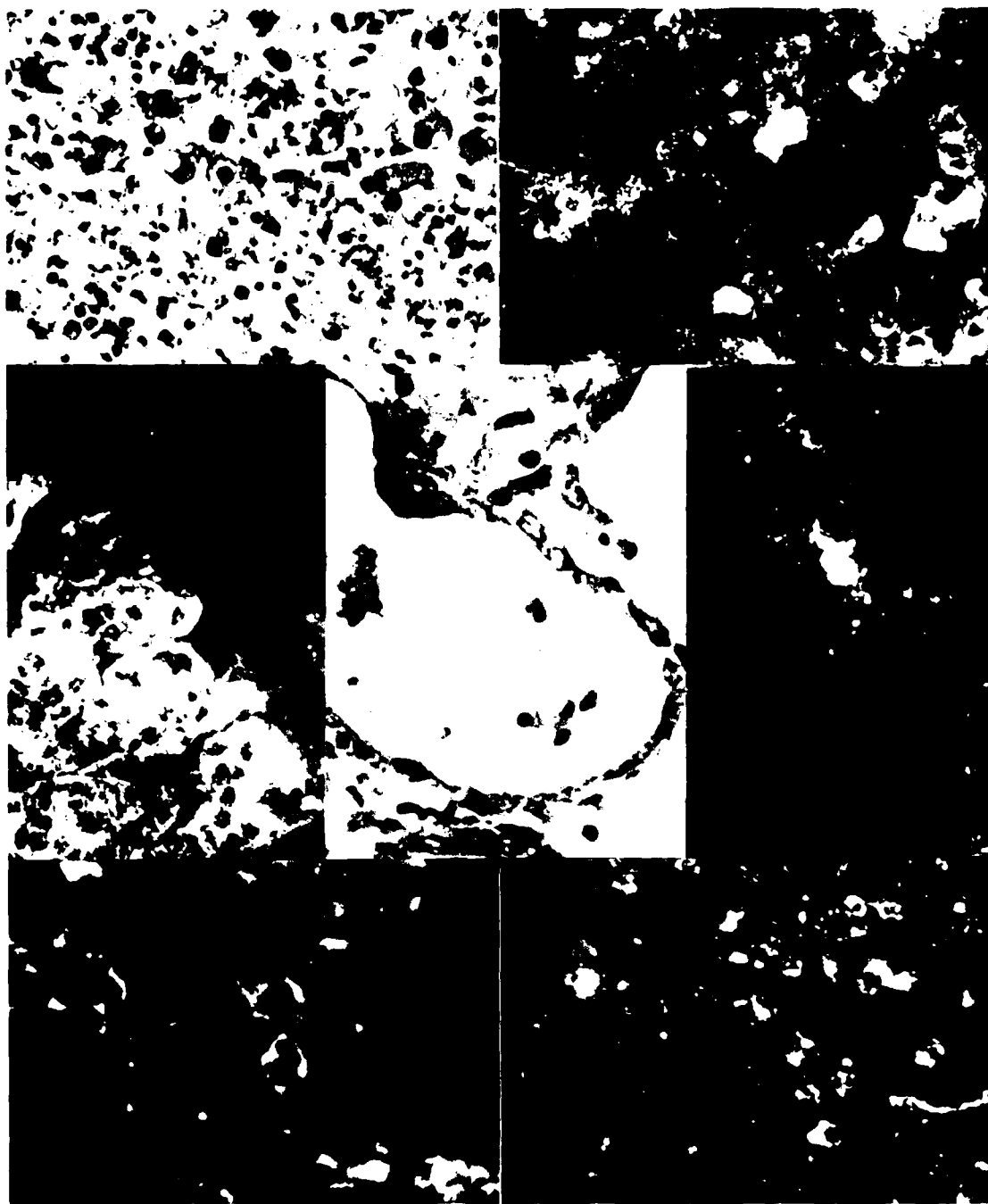
Figure 6. Tissues of rhesus monkeys after inoculation with Lassa virus. *Upper left,* liver 12 days after inoculation, with widespread hepatocellular necrosis and only a slight infiltration of inflammatory cells (hematoxylin and eosin, ×388). *Upper right,* liver 12 days after inoculation, with massive concentration of Lassa viral antigens in intact and degenerating hepatocytes (direct fluorescent antibody, ×412). *Middle left,* adrenal gland 13 days after inoculation, with Lassa viral antigens *present in epithelial cells of the zona glomerulosa and extending into the zona fasciculata* (direct fluorescent antibody, ×412). *Middle,* lung on day 12: alveolar septa are thickened and macrophages are free in the alveolar lumen (hematoxylin and eosin, ×823). *Middle right,* lung on day 12, with fine dustlike fluorescence in alveolar septa and intense fluorescence in occasional cells resembling alveolar macrophages (direct fluorescent antibody, ×604). *Lower left,* axillary lymph node 13 days after inoculation, with intense fluorescence concentrated in large cells immediately adjacent to germinal centers (direct fluorescent antibody, ×805). *Lower right,* spleen 13 days after inoculation: intense fluorescence is concentrated in large cells distributed throughout the red pulp, while the white pulp is essentially devoid of Lassa viral antigens (direct fluorescent antibody, ×412).

Discussion

The experimental infection of rhesus monkeys with Lassa virus appears to be a reasonable model for the human disease. Lassa virus infection of both humans and rhesus monkeys produces a systemic disease involving most visceral organs but sparing the central nervous system. In both humans and rhesus monkeys, the severity of infection correlates with viremia titers, elevations of transaminase levels in the blood, and fever. At the dosage levels used in the present study, ribavirin limited the development of viremia titers in treated monkeys to the lower values observed for surviving controls; similarly, the appearance of transaminases in the serum of treated monkeys was suppressed. The protective effect of ribavirin was greater in monkeys treated initially at the time of infection than in those treated initially on day 5. Thus it is reasonable to predict that ribavirin would be of value in treating persons soon after a known exposure to Lassa virus (for example, laboratory accidents, overt clinical exposures to blood, or contamination with patient excretions or secretions). Ribavirin might also be of value in treating patients who are first seen after onset of clinical signs, provided treatment is initiated early in the disease course. The efficacy of treatment with ribavirin initiated later than day 5 after viral inoculation in rhesus monkeys remains to be tested. The use of ribavirin in the treatment of human Lassa fever has not been reported.

Management of patients with Lassa fever has depended on supportive therapy and occasional attempts to produce passive immunity by administering immune plasma [2-4]. The number of patients treated with immune plasma is small, the clinical responses have been variable, and almost no virologic or immunologic parameters have been measured. To evaluate immunotherapy of Lassa infections more thoroughly, it will be reasonable to initiate such studies in monkeys, now that basic information for untreated infection with Lassa virus in monkeys is available; furthermore, the combined use of ribavirin and immune plasma might prove to be superior to either treatment alone, and should also be tested.

Although elucidation of the pathophysiology of Lassa viral infection in primates was not the primary purpose of this work, the data obtained do offer some insight into this question and help to explain why ribavirin was effective in treat-ment. Lassa virus was observed to be pantropic, causing lesions and probably dysfunction of multiple organ systems and resulting in a general toxemia. However, the central nervous system was spared. This was fortuitous, because ribavirin has been reported to be inactive against many viral infections of the central nervous system, unless the drug is administered directly into the brain [17]. After im inoculation of rhesus monkeys, ribavirin was concentrated in the same organs shown in figure 5 to be major sites of Lassa viral replication but was not concentrated in the central nervous system [18], yet no surviving monkeys developed any apparent neurologic sequelae during the four to seven months of observation after infection. It was feared that most or all monkeys that were spared an acute death from Lassa infection would develop neurologic signs and die several months after infection, as has been documented for infections with Machupo virus in primates [19, 20]. Fortunately, recognizable neurologic complications did not occur with Lassa fever in these nonhuman primates, and on this basis such complications would not be anticipated in humans with Lassa infections treated with ribavirin or immune plasma.

The apparent blind condition in two ribavirin-treated monkeys was disturbing and unexplained. Ophthalmologic examinations were not attempted because of the restrictions imposed by the design of the maximal containment system. Four months after the termination of this project, the monkeys were killed, and histologic examination of the eyes revealed no morphologic basis for the transient blindness. It is unlikely that ribavirin treatment itself was responsible for this condition. A similar transient blindness has been observed in two rhesus monkeys, never treated with ribavirin, that were recovering from an infection with Machupo virus, the agent of Bolivian hemorrhagic fever, and an arenavirus closely related to Lassa virus (G. A. E., unpublished observations). Similarly, two instances of transient blindness have been recognized in human patients who were recovering from Lassa virus infection who had not been treated with ribavirin (J. B. McCormick, personal communication). In one of those cases, in which an ophthalmologic examination was performed, the blindness was unilateral, characterized by a poor light reflex and increased opacity of the vitreous but no morphologic evidence of retino-pathy.

The simultaneous occurrence of viremia and antibody detected by the IFA technique suggested the possibility of circulating complexes of infectious virus and antibody. A detailed investigation of the humoral and cellular immune responses to Lassa virus infection is needed. This assessment should provide critical insight into such diverse areas as the pathogenesis of Lassa infection, arenavirus taxonomy, diagnosis of infection, evaluation of immunotherapy regimens, and the interpretation of serologic responses to experimental vaccines of Lassa virus.

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